CRI/ICEIT
NEWSLETTER

VOL. 20 NO. 2 – April 2010
ISSN 0858-2793
BANGKOK, THAILAND

Chulabhorn Research Institute

INTERNATIONAL CENTRE FOR ENVIRONMENTAL AND INDUSTRIAL TOXICOLOGY (ICEIT)

CRI's ICEIT has been designated as a
"UNEP Centre of Excellence for Environmental and Industrial Toxicology".

Health Effects of Maternal Exposure to Air Pollutants During Pregnancy

Many studies have reported an association between maternal exposure to air pollutants, and possibly traffic-related air pollutants during pregnancy and the influence on fetal growth.

Traffic-related air pollution is a mixture of thousands of compounds present in gaseous form or as particulate matter (PM). These include aromatic hydrocarbons (e.g., benzene, polycyclic aromatic hydrocarbons), nonaromatic hydrocarbons (e.g., alkanes, olefins), metals, and inorganic gases such as nitrogen oxides and carbon monoxide. Most studies have focused on carbon monoxide, nitrogen dioxide, PM, and polycyclic aromatic hydrocarbons. With a few exceptions, exposure estimates were based on environmental models of outdoor air pollution levels close to the home address. These do not take into account the fact that outdoor levels of specific pollutants do not always reflect indoor levels, exposure in the workplace, and, importantly, levels in transit, which corresponds to a significant proportion of total personal exposure. Therefore, studies relying on a personal exposure assessment are warranted.

For a few traffic-related air pollutants, animal experiments have reported effects of maternal exposure on fetal growth. In rodents, airborne benzene exposure during pregnancy induces a reduction in fetal weight. In humans, studies of associations between benzene levels and pregnancy outcome have been conducted only in occupational settings, where benzene exposure is probably correlated with other chemicals than in the general population. Because of its antiknocking properties, benzene is used as an additive in gasoline; its presence in the atmosphere is attributable to industrial emissions and, predominantly, to motor vehicle traffic and combustion processes. Overall, the main sources of exposure in the general population are tobacco smoke, traffic, and other combustion processes. For these reasons, benzene monitoring is a relevant candidate as a proxy measure of exposure to air pollutants related to traffic and to gasoline uses; moreover, it can be assessed by passive air samplers, which are light and relatively simple.

In studies on effects of air pollutants, fetal growth has most often been assessed by measures of birth weight, taking into account gestational duration. One study reported a negative association between personal exposure to fine PM (aerodynamic diameter $\leq$ 2.5 µm; $PM_{2.5}$) and head circumference, and another reported a possible effect of air pollution levels in early pregnancy on fetal ultrasound measurements, including head circumference. Ultrasound measures constitute a promising approach to examine how early air pollution effects manifest in fetal growth. Studying head size is particularly important, as head size is a marker of fetal growth that may specifically be associated with cognitive development in childhood.

Now a new study has been conducted with the aim of assessing the relation between maternal personal exposure to airborne benzene during pregnancy and fetal growth. This study was conducted in a subgroup of the EDEN (study of pre- and early postnatal

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determinants of the child's development and health) mother-child cohort.

Women at over 20 gestational weeks (weeks of amenorrhea) were recruited from the maternity wards in two French maternity hospitals between September 2003 and January 2006.

A subsample of 271 nonsmoking women carried a diffusive air sampler for a week during the 27th gestational week, allowing assessment of benzene exposure. Head circumference of the offspring was estimated by ultrasound measurements during the second and third trimesters of pregnancy and birth.

Median benzene exposure was 1.8 µg/m^3 (5th, 95th percentiles, 0.5, 7.5 µg/m^3). Log-transformed benzene exposure was associated with a gestational age-adjusted decrease of 68 g in mean birth weight [95% confidence interval (CI), –135 to –1 g] and of 1.9 mm in mean head circumference at birth (95% CI, –3.8 to 0.0 mm). It was associated with an adjusted decrease of 1.9 mm in head circumference assessed during the third trimester (95% CI, –4.0 to 0.3 mm) and of 1.5 mm in head circumference assessed at the end of the second trimester of pregnancy (95% CI, –3.1 to 0.0 mm).

This prospective study among pregnant women is one of the first to rely on personal monitoring of exposure; a limitation is that exposure was assessed during 1 week only. Maternal benzene exposure was associated with decreases in birth weight and head circumference during pregnancy and at birth. This association could be attributable to benzene and a mixture of associated traffic-related air pollutants.

Source: Environmental Health Perspectives, Vol. 117, No. 8, August 2009.

PULMONARY EFFECTS OF INHALED DIESEL EXHAUST IN AGED MICE

One of the most sensitive populations to the adverse health effects of inhaled particulate matter (PM) is the elderly, typically individuals over 65 years of age. Aging is associated with deterioration of both innate and adaptive immunity. Most notable changes include age-related deficits in macrophage and neutrophil activity which are thought to underlie increased susceptibility of the elderly to respiratory infections. Alterations in lung permeability and mechanics have also been described in elderly humans and rodents. These changes may also contribute to the increased sensitivity of older individuals to the adverse effects of inhaled pollutants.

As oxidative stress appears to be a common mechanism underlying the biological effects of PM and other air pollutants, a particularly important contributory factor may be age-related alterations in lung antioxidants and intracellular oxidative stress. These include reduced levels of superoxide dismutase (SOD), vitamin C and dysregulation of vitamin B12 and folic acid metabolism in the lung. Aging is also associated with aberrant generation of reactive oxygen and nitrogen species by macrophages resulting in oxidative/nitrosative stress.

The present studies analyzed potential mechanisms underlying increased susceptibility of the elderly to PM using diesel exhaust (DE) as a model. Mice (2 m and 18 m) were exposed to DE (0, 300, and 1000 µg/m^3) for 3 h once (single) or 3 h/day for 3 days (repeated). Bronchoalveolar lavage fluid (BAL), serum and lung tissue were collected 0 and 24 h later. Exposure to DE resulted in structural alterations in the lungs of older but not younger mice, including patchy thickening of the alveolar septa and inflammatory cell localization in alveolar spaces. These effects were most pronounced 24 h after a single exposure to the higher dose of DE. Significant increases in BAL nitrogen oxides were also noted in older mice, as well as expression of lipocalin 24p3, an oxidative stress marker in the lung with no effects in younger mice. Following DE inhalation, expression of Tumor Necrosis Factor alpha (TNFα) was upregulated in lungs of both younger and older mice; however, this was attenuated in older animals. Whereas exposure to DE resulted in increases in lung Interleukin-6 (IL-6) expression in both older and younger mice, IL-8 increased only in older animals. In younger mice, constitutive expression of manganese superoxide dismutase (MnSOD) decreased after DE exposure, while in older mice, constitutive MnSOD was not detectable and DE had no effect on expression of this antioxidant.

The present studies demonstrate that single or repeated inhalation of DE results in significant structural and inflammatory changes in the lungs of older but not younger mice. However, no consistent differences were noted between the two exposure protocols, suggesting that the impact of diesel is rapid. Alternatively, more prolonged exposures may be required for differences in the effects of single and repeated DE exposures to be observed. It should be noted that the exposure doses used in our studies are relevant to human exposure in occupational and environmental settings. Occupational exposures to DE can exceed 1000 µg/m^3, and levels of PM in the world's largest cities have been shown to exceed 300 µg/m^3. The data presented in these studies showing altered production of inflammatory mediators and reduced MnSOD in lungs of older mice suggest a potential mechanism for the increased susceptibility of the elderly to inhaled PM. However, at present we cannot exclude the possibility that some of these biological effects are due to different levels of nitrogen oxides (NOx) and carbon oxides (COx) in the DE. Identification of key inflammatory mediators involved in the response of older mice to DE may help in the design of new and effective approaches to mitigating pulmonary pathology in the geriatric population.

Source: Toxicology and Applied Pharmacology, Vol. 241, Issue 3, December 2009.

A Study of Occupational Exposure to 1,4-Dichlorobenzene

The chemical 1,4-dichlorobenzene (1,4-DCB) is extensively used in industry and households. In industry, it is used as a chemical intermediate for dyes, polyphenylene sulfide resin, and 1,2,4-trichlorobenzene. In households and public lavatories, it is used as a room deodorant, toilet deodorizer, moth repellent, and mildew control agent. From 1998 to 2002, annual production of 1,4-DCB in the United States increased from 23,000 to 45,000 metric tons.

1,4-DCB sublimes easily, so that 1,4-DCB exposure occurs mainly by inhalation. Exposure to 1,4-DCB by inhalation has been extensively evaluated in homes, factories, and new buildings. People who inhale 1,4-DCB excrete 2,5-dichlorophenol (2,5-DCP), the major metabolite of 1,4-DCB, in urine. Urinary 2,5-DCP can be divided into two types, free form and conjugated form, and is a good biological marker of 1,4-DCB exposure.

The adverse health effects of 1,4-DCB have been extensively studied in animals, and numerous animal studies have shown that 1,4-DCB may produce hematological abnormalities and impair kidney and liver function.

There are very few studies, however, describing the adverse effects of 1,4-DCB in humans, even though animal models demonstrate that 1,4-DCB exposure may have adverse effects on blood components, liver, and kidney.

Now, a new study has been carried out to explore the health effects of exposure in workers involved in the manufacture of mothballs containing 1,4-DCB and to evaluate the correlation between urinary 2,5-DCP concentration and hematological, hepatological, and renal function.

This cross-sectional study was conducted from May 2003 to July 2004 in Taiwan and recruited 60 volunteer workers working in plants in northern Taiwan making mothballs containing mostly 1,4-DCB. The non-exposed, control group was medical workers and administrative personnel. In addition, the number of working years was not significantly different for exposed and non-exposed workers. This study thus included 46 1,4-DCB-exposed workers and 29 non-exposed workers. For exposed workers, the procedure of mothball manufacture included four steps: pulverization of the raw 1,4-DCB (purity > 99.5%), addition with mixing of perfume and color additives, pressing into blocks, and filling and packaging in plastic bags or containers.

Before the study began, an interviewer collected essential information using a standardized questionnaire and presented the study objective. The information included participant characteristics (age, gender, weight, height, etc.), level of education, work experience (e.g. seniority; how many years they have worked), work habits, personal medical history, self-reported symptoms (including irritation of the nose, throat, eyes, and skin), dietary habits, and mothball use at home. The questionnaire distinguished between three levels: cigarette smoking, drinking alcohol (including the amount of daily alcohol consumption), and betel nut chewing. Ex-smokers, ex-drinkers, and ex-chewers were smokers, drinkers, and chewers who had stopped at least 2 years before participation in the study.

All blood and urine samples were collected midweek in the morning. The samples were collected in the morning because the biological residence time of 1,4-DCB in human tissue is 20-30 h. Therefore, workers had to be exposed to 1,4-DCB at least 1 or 2 days or else its metabolites would be excreted out of the body. Moreover, measurement of urinary metabolites could be an indicator of recent exposure, since 1,4-DCB can be excreted several days post-exposure. Subjects were instructed to collect their clean-catch midstream urine samples in clean glass bottles and then to tightly seal the bottles with caps composed of polyketone for preventing absorption of 2,5-DCP. Samples were transported at 4ºC, and stored at –20ºC until analysis of 2,5-DCP. Blood samples were drawn by nurses. All tubes were transported to the laboratory immediately and kept at 4ºC until analysis. Hematological analysis included white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT) count. Liver function and kidney function analyses included assays for blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), and alanine amino-transferase (ALT).

Urinary 2,5-DCP was analyzed using a novel method for determining both 1,4-DCB (parent compound) and free 2,5-DCP.

The results of the study showed urinary 2,5-DCP concentration, WBC count, and serum ALT level were higher in exposed workers than in non-exposed ones. Furthermore, the WBC count and ALT level were significantly correlated with the concentration of 2,5-DCP in urine. The BUN was significantly higher in on-site exposed workers. Urinary 2,5-DCP concentration was significantly lower in workers who wore personal protective equipment (PPE) during work than in those who did not. The higher urinary 2,5-DCP concentration in exposed (105.38 µg/L) than non-exposed (1.08 µg/L) workers suggests that 1,4-DCB exposure may increase the 2,5-DCP concentration in urine. Moreover, exposure to 1,4-DCB may also increase WBC count and ALT activity, and PPE may protect workers from 1,4-DCB exposure.

Source: International Archives of Occupational and Environmental Health, Vol. 82, No. 9, October 2009.

A New Study Tests the Hypothesis that Exposure to Whole Diesel Exhaust Enhances Angiogenesis/Vasculogenesis

Epidemiologic studies have shown a strong link between airborne particulate matter (PM) exposure and cancer morbidity and mortality. Although multiple mechanisms have been proposed, few studies have mechanistically evaluated the impact of diesel exhaust, primarily generated from motor vehicle sources, on the angiogenesis that cancers are dependent upon for their growth.

Angiogenesis constitutes physiological and pathological responses to ischemia. For multicellular organisms to grow beyond their sizes, they must recruit new blood vessels by vasculogenesis and angiogenesis. Therefore, vasculogenesis and angiogenesis play a key role in the development of tumors. Recently, a growing body of epidemiological and clinical evidence has led to a heightened concern about the potential deleterious effects of ambient air pollution on health and its relation to systemic diseases, such as cardiovascular diseases. These associations are the strongest for fine and ultrafine particulate air pollutants, of which the combustion-derived PM in diesel exhaust particle (DEP) is an important component. However, to date, there has been no report about the effect of diesel exhaust exposure on angiogenesis and vasculogenesis, especially at an environmentally relevant exposure level.

Thus a new study has been carried out. To investigate if the vasculogenic/angiogenic effect of exposure to whole diesel exhaust (WDE) in the initiation of the angiogenesis/vasculogenesis (other than progression of tumor) to diesel exhaust was via hypoxia, two ischemic models were designed for the study. One was created by the ligation of femoral artery and vein, which is a clinically extreme hypoxia situation. Another model was based on implantation of scaffold into the dorsum of the mouse, which was intended to mimic the very low-grade inflammation and hypoxia that human body/tissues may encounter to traffic corridor exposure where high WDE levels occur. Through these two clinically relevant models, researchers examined the effects of WDE exposure on angiogenesis and vasculogenesis under both extreme hypoxia and very mild hypoxic circumstances. The study intended to investigate that diesel exhaust induced angiogenesis and vasculogenesis *in vivo* in a mouse model of apolipoprotein E-deficient ($ApoE^{-/-}$) mice and vasculogenesis *in vitro*. This murine model, along with *in vitro* vasculogenesis assay, provides insights into the mechanisms responsible for air pollution-induced angiogenesis and vasculogenesis.

The two *in vivo* models, i.e. scaffold implantation with endothelial cells and hindlimb ischemia, were designed to provide two extremely hypoxic conditions that occur in human bodies in real world. The major findings in a murine model of hindlimb ischemia exposed by inhalation to WDE for up to 8 weeks, along with *ex vivo* aortic ring and *in vitro* endothelial cell culture experiments are the following: (1) WDE exposure significantly increased inflammatory cell infiltration in the tissues and scaffolds; (2) WDE exposure induced vasculogenesis, which was manifested by increased CD31 and α-smooth muscle actin (α-SMA) expression in the scaffolds; (3) WDE exposure resulted in decreased endothelial nitric oxide synthase (eNOS) expression, which may lead to hypoxia by decreasing the vascular production of nitric oxide; (4) WDE exposure enhanced functional vascular blood flow, and induced capillary tube formation and capillary sprouting. Inflammation provides a potential mechanistic link between PM air pollution exposure and adverse health effects observed in epidemiologic studies, yet considerable uncertainty remains as to whether inflammation is mediating these effects, and where that inflammation is occurring. In the current study, hematoxilyn and eosin staining was performed to measure the total amount of cells in the scaffolds. Since the same amount of endothelial cells was seeded in each scaffold (1 x 10^6 cells), the difference between WDE and filtered outdoor air (FA) groups should be due to either the increase in inflammatory cell infiltration, or the vasculogenic effect (endothelial proliferation). The transient increase of total cells in WDE group at week 2 in the study, which was coupled with significant increase of CD31 staining, but no increase of macrophages, indicates that there was no significant increase of inflammatory cell infiltration at week 2 induced by WDE exposure. At week 8, a significant increase in macrophages in the scaffolds in the WDE group was seen, indicating that PM exposure-induced inflammation has "low-grade" characteristics, and is not an acute event, which is consistent with other reports. PM air pollution has been shown to be associated with several adverse cardiovascular health outcomes, and patients with diabetes may be especially vulnerable. One potential pathway is via inflammation and endothelial dysfunction. In one previous study, $PM_{2.5}$ exposure in subjects with type 2 diabetes mellitus showed consistent association with increased inflammatory markers, suggesting that inflammatory mechanism may explain the increased risk of air pollution-induced cardiovascular events. To study the effects of DEP on pulmonary and systemic inflammatory responses, another study in mice using LPS challenge found that DEP exacerbated pulmonary inflammation and vascular permeability,

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ASSOCIATION BETWEEN SERUM PERFLUOROCTANOIC ACID AND THYROID DISEASE IN THE GENERAL POPULATION OF THE UNITED STATES

The perfluoroalkyl acids (PFAAs) are a family of synthetic, highly stable perfluorinated compounds with a wide range of uses in industrial and consumer products, from stain and water-resistant coatings for carpets and fabrics to fast-food contact materials, fire resistant foams, paints and hydraulic fluids. The carbon-fluoride bonds that characterize PFAAs and make them useful as surfactants are highly stable and recent reports indicate the widespread persistence of certain PFAAs in the environment and in wildlife and human populations globally. Two of the PFAAs of most concern are the eight carbon chained perfluoroctane sulphonate (PFOS) and perfluorooctanoic acid (PFOA, 'C8').

Most persistent organic pollutants are lipophilic and accumulate in fatty tissues, but PFOS and PFOA are both lipo- and hydrophobic, and following absorption will bind to proteins in serum rather than accumulating in lipid. The renal clearance of PFOA and PFOS is negligible in humans, leading to reported half lives in blood serum of 3.8 and 5.4 years for PFOA and PFOS respectively. Human biomonitoring of the general population in various countries has shown that in addition to the near ubiquitous presence of PFOS and PFOA in blood, they may also be present in breast milk, liver, seminal fluid and umbilical cord blood.

Extensive laboratory studies of the toxicology of PFOA and PFOS have reported enlargement of the liver, modulation of sex hormone homeostasis, developmental and immune system toxicity, hypolipidemia and reduced body weight in rodent and non-human primate models. Research interest has focused on the ability of these compounds to bind to nuclear receptors including the peroxisome proliferator activating receptor (PPARα), and to disrupt serum protein ligand binding, highlighting PFOA and PFOS as potential endocrine disruptors.

Endocrine systems that may be targets of endocrine disrupting chemicals include the hypothalamus-pituitary-thyroid axis (HPT). Thyroid hormone is essential for the normal physiological function of nearly all mammalian tissues. Thyroid hormone status is controlled by a well-established feedback mechanism, in which thyroid stimulating hormone (TSH) stimulates the thyroid to synthesize T4, which is then converted to the biologically active T3. The rate of release of TSH is regulated by the hypothalamus as well as by the circulating levels of T3 and T4. Therefore, multiple physiological steps including hormone biosynthesis, transport, metabolism or action on target cells are required for thyroid hormone homeostasis.

Numerous studies have now shown PFAAs to impair thyroid hormone homeostasis in animal studies. Depression of serum T4 and T3 has been reported by several authors in PFOS-exposed rats, without the concomitant increase in TSH that would be expected through feedback stimulation. Earlier mechanistic studies of the structurally related compound perfluorodecanoic acid (PFDA) showed that it could reduce serum thyroid hormone levels by apparently reducing the responsiveness of the HPT axis and by displacing circulating thyroid hormones from their plasma protein binding sites. Whilst circulating hormone levels were depressed, the activities of thyroid hormone sensitive liver enzymes were elevated, suggesting that functional hypothyroidism was not occurring. A similar mechanism for PFOS has been hypothesized. A recent study of the mechanisms involved in PFOS-induced hypothyroxinemia in rats has indicated that increased conjugation of T4 in the liver, catalysed by the hepatic enzyme uridine diphosphoglucuronosyl transferase (UGT1A1), and increased thyroidal conversion of T4 to T3 by type 1 deiodinase may be partly responsible for the effects seen. Taken together, these findings suggest that the effects of PFAAs on thyroid hormone physiology are multiple and complex.

Extrapolations from animal laboratory studies such as these to an estimation of the risks posed by PFOA and PFOS to thyroid function in humans are complicated by the extreme variations reported in their toxicokinetic profile between species. The extremely long half lives of PFOA and PFOS in humans are in contrast with the relatively rapid elimination seen in animal models: the serum half life of PFOS in rats is around 100 days, drawing attention to the potential risks to human health. Disruption to thyroid hormone balance was not found in previous studies of community exposure to PFOA or PFOS. Modest associations between PFOA and thyroid hormones (negative for free T4 and positive for T3) were reported in 506 PFOA production workers across three production facilities. There were no associations between TSH or T4 and PFOA and

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A New Study Tests the Hypothesis that Exposure to Whole Diesel Exhaust Enhances Angiogenesis/Vasculogenesis

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and increased fibrinogen and E-selectin levels, suggesting that adverse health effects of PM air pollution occur in sensitive populations with predisposing vascular and/or pulmonary disorders, including ischemic vascular diseases and respiratory infection.

As currently understood, neovascularization is the result of several processes, including angiogenesis, arteriogenesis, and vasculogenesis. The term angiogenesis describes the sprouting of new capillaries from postcapillary venules, and in adults, it is stimulated mainly by tissue hypoxia. Angiogenesis leads predominantly to the development of capillaries, although the formation of larger-size vessels has also been noted in certain animal models. In contrast, arteriogenesis refers to the process of maturation or perhaps *de novo* growth of collateral conduits that are frequently of a sufficient diameter to be visualized angiographically. Vasculogenesis is the process of an *in situ* formation of blood vessels from circulating endothelial progenitor cells and vascular progenitor cells.

This present study demonstrates that WDE exposure increases inflammatory cell infiltration in the tissues, enhances vessel volume and blood flow, and increases capillary tube formation and capillary sprouting, thereby inducing angiogenesis and vasculogenesis via increasing hypoxia-inducible factor-1 (HIF-1) and vascular endothelial growth factor (VEGF) expression and decreasing prolylhydroxylase2 (PHD2) expression. These findings may have significant impact on our understanding of vehicular traffic-related air pollution, especially PM, on long-term human health effects, especially tumor initiation and development.

Source: Toxicology Letters, Vol. 191, Issue 1, December 2009.

ASSOCIATION BETWEEN SERUM PERFLUOROCTANOIC ACID AND THYROID DISEASE IN THE GENERAL POPULATION OF THE UNITED STATES

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the free hormone levels were within the normal reference range.

Given the evidence from animal studies of thyroid hormone imbalance and the varied epidemiological results from community and occupational exposures, a new study has aimed to explore the hypothesis that higher serum PFOA and PFOS concentrations would be associated with thyroid disease in the general adult population. The U.S. Centers for Disease Control and Prevention (CDC) environmental chemical biomonitoring program, using samples from the US National Health and Nutrition Examination Survey (NHANES), provides large scale data on serum PFAA concentrations in population representative samples. In the present study, researchers use these data to estimate associations between PFOA/S concentrations and thyroid disease in representative samples of the general population of the USA.

The prevalence of thyroid disease is markedly higher in women than in men, and therefore sex specific associations have been estimated. The study shows that across all the available data from NHANES, thyroid disease associations with serum PFOA concentrations are present in women and are strongest for those currently being treated for thyroid disease. For men, a near significant association between PFOA and treated thyroid disease was also present: an interaction term analysis suggests that the PFOA trends in men and women are not significantly different, despite the relative rarity of thyroid disease in men. In addition, a nominally significant association was present between PFOS concentrations and treated thyroid disease in men, but not in women.

The presence of associations with both PFOA and PFOS raises the issue of how best to perform risk assessments for combinations of perfluorochemicals. The somewhat divergent risk patterns for the two compounds supports their separate risk assessment, given that current legislative advice is to consider the combined effects of chemicals only when two or more chemicals in a mixture affect the same tissue, organ or organ system.

The results are important because PFAAs are detectable in virtually everyone in society with ubiquitous presence across global populations.

Further work is clearly needed to characterize the PFOA and PFOS associations with specific thyroid diagnoses and thyroid hormone levels in the general population, and clarify whether the associations reflect pathology, changes in exposure or altered pharmacokinetics. Longitudinal analyses are also needed to establish whether high exposures predict future onsets of thyroid disease, although concurrent alteration of thyroid functioning would still be a cause for concern.

Higher PFOA and PFOS concentrations are associated with thyroid disease (and being on thyroid related medication) in the NHANES US general adult population representative study samples. More work is needed to establish the mechanisms underlying this association and to exclude confounding and pharmacokinetic explanations.

Source: Environmental Health Perspective, published online ahead of print on January 20, 2010.

Health Risks from the Use of Organophosphorus Insecticides in Thai Farming Communities

The World Health Organization classifies methyl parathion as an "Extremely Hazardous (Class Ia) Pesticide". Like other types of organophosphorus insecticides, it inhibits acetylcholinesterase (an enzyme in the central nervous system), resulting in an accumulation of acetylcholine at nerve endings. Excess acetylcholine at synaptic junctions in neurons causes weakness and fasciculation and disrupts normal neural transmission. Acute exposure to organophosphorus compounds may cause loss of consciousness, dizziness, confusion, headaches, difficult breathing, chest tightness, wheezing, vomiting, diarrhea, cramps, tremors, blurred vision, sweating, and death. Although studies on health problems associated with chronic, low-level exposure to methyl parathion are limited, long-term exposure has been associated with neuropsychiatric disorders and serum cholinesterase suppression.

After being introduced into the environment, methyl parathion can remain intact, or it can be environmentally transformed into methyl paraoxon. Both can be hydrolyzed in the environment to form dimethylthiophosphate (DMTP) or dimethylphosphate (DMP) and paranitrophenol (PNP). The most common way of assessing exposure to methyl parathion and its oxon is to measure its "selective" urinary metabolite, PNP, the metabolite formed from the moiety on the pesticide, which is attached to the O,O-dimethylphosphothionate group. PNP is a metabolite of the pesticides methyl parathion, parathion, O-ethyl 4-nitrophenyl phenylphosphonothioate (EPN), and potentially other industrial precursors.

In addition to PNP, parathion and methyl parathion and their respective oxons are also metabolized through oxidation and/or dealkylation processes to form dialkylphosphate (DAP) metabolites. For methyl parathion, the metabolic end products are DMP and DMTP; parathion produces diethylphosphate and diethylthiophosphate. Although parathion and methyl parathion form the same "selective" metabolite-PNP-they could be differentiated based on the formation of their DAP metabolites. Although many organophosphorus insecticides can be metabolized to DAPs, the combination of the selective metabolite and DAP metabolite data might help us understand the source of the PNP.

Because of their high toxicity, parathion and methyl parathion were banned from agricultural manufacture, import, and use in Thailand in 1988 and 2004, respectively. Although Thai farmers still use EPN, during the past decade it was imported less frequently into the country than methyl parathion. From 1996 to 2003, methyl parathion was one of the top 20 imported pesticides.

Before the recent agricultural ban of methyl parathion, a study found that a sample of Thai farmers and children in Chiang Mai province in 2003 had measureable levels (100% and 98% frequencies of detection, respectively) of PNP, suggesting widespread exposure. After the ban on methyl parathion, a marked decrease of PNP in Thai urinary samples collected was anticipated; however, data from two post-ban studies suggested otherwise. Now a new study has been conducted to evaluate the correlation among methyl parathion-related metabolites in these two post-ban studies to assess whether urinary PNP, as measured as part of a Thai study conducted after the methyl parathion ban, resulted from post-ban methyl parathion exposure.

This biological monitoring study was carried out in Chiang Mai, Thailand, and measured PNP and DAP metabolites of methyl parathion in urine samples collected from 136 farmers (age 20 to 65 years) and 306 school children (age 10 to 15 years) in 2006. Participants came from two topographically different areas: one was colder and mountainous, whereas the other was alluvial with climate fluctuations depending on the monsoon season. Both children and farmers were recruited from each area. Despite methyl parathion's prohibited use in agriculture in 2004, researchers detected PNP in >90% of all samples analyzed. A nonparametric correlation test (PNP vs. DMP and DMTP) was applied to determine whether the PNP found in most of the samples tested resulted from exposures to methyl parathion. DMP (Spearman's rho = 0.601 for farmers and Spearman's rho = 0.263 for children) and DMTP (Spearman's rho = 0.296 for farmers and Spearman's rho = 0.304 for children) were positively correlated with PNP, suggesting a common source for the three analytes, presumably methyl parathion or related environmental degradates. Although researchers found a modest correlation between the metabolites, the findings suggest that despite the prohibition, at least a portion (approximately 25% to 60%) of the PNP detected among farmers and children in Thailand may be attributed to exposure from continued methyl parathion use.

Source: Archives of Environmental Contamination and Toxicology, Vol. 57, No. 3, October 2009.

PRENATAL PHTHALATE EXPOSURE AND REDUCED MASCULINE PLAY IN BOYS

Phthalate esters are pervasive environmental chemicals. Although several of these are now banned for use in toys and some other products designed for young children, this legislation does not limit prenatal exposure. Moreover, phthalates are present in so many other products and manufactured in such quantity, that exposure is virtually universal. A large body of work in laboratories around the world has demonstrated that, in experimental animals, when exposure occurs during the period of foetal sexual differentiation, some phthalates, notably di(2-ethylhexyl) phthalate (DEHP) and dibutyl phthalate (DBP), inhibit the synthesis of testosterone by Leydig cells, thereby reducing foetal testosterone concentration. As a result, male pups exhibit a cluster of altered androgen dependent anatomical features that reflect disordered sex differentiation, including a reduced – that is, a less masculine – anogenital distance (AGD), impaired testicular descent and reduced genital size. This cluster of alterations has been referred to as the 'phthalate syndrome'. Some phthalate-related changes have also been identified in adult female rodents, but no significant changes have been reported in female neonates. In male rodents, the phthalate syndrome, which is initially identified neonatally, has been shown to have adverse consequences for later sexual development. Researchers recently reported data demonstrating an association between prenatal exposure in humans, particularly to DEHP and its urinary metabolites, and a similar cluster of reproductive developmental outcomes in male infants. In addition, free serum testosterone in human male infants has been negatively correlated with levels of some phthalate metabolites in breast milk. However, the long-term consequences of these findings for humans are uncertain.

In particular, the potential for these antiandrogens to influence the course of brain sexual differentiation has only recently been addressed. This question is rooted in the understanding of how gonadal hormones influence mammalian neural development. Testosterone exposure during early development produces a masculine neural phenotype by influencing cell survival, neural growth and neurochemical specification. In rodents, this process involves the enzyme aromatase, which, by a biological irony, converts testosterone into oestradiol, which then shapes the male structure. In rats, the critical programming window for genital tract development occurs in gestational days 18-21, a period that corresponds to a testosterone surge in the developing male. In humans, the testes begin to function at about week 8 of gestation and, while dates are uncertain, testosterone appears to be elevated in the male foetus from about weeks 8 to 24 of gestation. The critical period for brain sexual differentiation is unknown and may not be the same as that for reproductive tract development. Whatever the critical period, testosterone is an essential mediator; if the antiandrogenic actions of phthalates reduce its secretion by the foetus, brain sexual differentiation may be altered. Play behaviours offer themselves as a test of the hypothesis that phthalate exposures during gestation may alter brain sexual differentiation and its behavioural outcomes.

Young male and female humans, rats and non-human primates all show sex differences in play behaviours. Young male rats and non-human primates, for example, engage in more play-fighting or rough-and-tumble play than their female counterparts. Young male rhesus monkeys, like boys, also show distinct preferences for toys with wheels and vervet monkeys show sex differences in toy preferences similar to those shown previously in children. Finally, and more central to the researchers' hypothesis, standardized inventories of sex differences in play behaviours have been constructed, such as the Pre-School Activities Inventory (PSAI), which has been shown to be sensitive to early androgen exposure and to reflect the endocrine-disrupting properties of dioxins and polychlorinated biphenyls (PCBs).

To assess play behaviours in relation to phthalate metabolite concentrations in prenatal urine samples, researchers conducting the present study recontacted participants in the Study for Future Families whose phthalate metabolites had been measured in mid-pregnancy urine samples. The Study for Future Families is a multi-centre pregnancy cohort study in which women and their partners were recruited at prenatal clinics. Initial recruitment took place at clinics affiliated to university hospitals in Los Angeles, Minneapolis and Colombia between September 1999 and December 2002. Mothers completed a questionnaire including the PSAI, a validated instrument used to assess sexually dimorphic play behaviour. Researchers examined play behaviour scores (masculine, feminine and composite) in relationship to (log10) phthalate metabolite concentrations in mother's urine separately for boys (N = 74) and girls (N = 71). Covariates (child's age, mother's age and education and parental attitude towards atypical play choices) were controlled using multivariate regression models. Concentrations of dibutyl phthalate metabolites, mono-n-butyl phthalate (MnBP) and mono-isobutyl phthalate (MiBP) and their sum, were associated with a decreased (less masculine) composite score in boys (regression coefficients –4.53, –3.61 and –4.20, p = 0.01, 0.07 and 0.04 for MnBP, MiBP and their sum respectively). Concentrations of two urinary metabolites of mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and the sum of these DEHP metabolites plus mono-(2-ethylhexyl) phthalate were associated with a decreased masculine score (regression coefficients –3.29, –2.94 and –3.18, p = 0.02, 0.04 and 0.04 for MEHHP, MEOHP and the sum respectively). No strong associations were seen between behaviour and urinary concentrations of any other phthalate metabolites in boys, or between girls' scores and any metabolites. These data, although based on a small sample, suggest that prenatal exposure to antiandrogenic phthalates may be associated with less male typical play behaviour in boys. The findings suggest that these ubiquitous environmental chemicals have the potential to alter androgen-responsive brain development in humans.

Source: International Journal of Andrology, Vol. 33, Issue 2, October 2009.

The ICEIT NEWSLETTER is published quarterly by the International Centre for Environmental and Industrial Toxicology of the Chulabhorn Research Institute. It is intended to be a source of information to create awareness of the problems caused by chemicals. However, the contents and views expressed in this newsletter do not necessarily represent the policies of ICEIT.

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